Title

2-arachidonoyl glycerol potently induces cholecystokinin secretion in murine enteroendocrine STC-1 cells via cannabinoid receptor CB1

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Abbreviated title

2-arachidonoyl glycerol stimulates cholecystokinin secretion via CB1

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Keywords

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Abstract

Cholecystokinin (CCK) is a peptide hormone secreted from enteroendocrine cells and regulates the exocrine pancreas, gastric motility, and appetite. Dietary triacylglycerols (TG) are hydrolyzed to fatty acids (FA) and 2-monoacylglycerols (2-MAG) in the small intestine. Although it is well known that FA stimulate CCK secretion, whether 2-MAG have the CCK-releasing activity remains unclear. We examined the CCK-releasing activity of four commercially available 2-MAG in a murine CCK-producing cell line, STC-1, and the molecular mechanism underlying 2-MAG-induced CCK secretion. CCK released from the cells was measured using ELISA. Among four 2-MAG (2-palmitoyl, 2-oleoyl, 2-linoleoyl, and 2-arachidonoyl monoacylglycerols) examined, 2-arachidonoyl glycerol (2-AG) potently stimulated CCK secretion in a dose-dependent manner. Structurally related compounds, such as 2-arachidonoyl glycerol ether and 1-arachidonoyl glycerol, did not stimulate CCK secretion. Both arachidonic acid and 2-AG stimulated CCK secretion at 100 μM, but only 2-AG did at 50 μM. 2-AG-induced CCK secretion, but arachidonic acid-induced CCK secretion was attenuated by treatment with a cannabinoid receptor 1 (CB1) antagonist. These results indicate that a specific 2-MAG, 2-AG, directly stimulates CCK secretion via CB1.

Abbreviations

1-AG	1-arachidonoyl glycerol
2-AG	2-arachidonoyl glycerol
2-AG ether	2-arachidonoyl glycerol ether
2-LG	2-linoleoyl glycerol
2-MAG	2-monoacylglycerol(s)
2-OG	2-oleoyl glycerol
2-PG	2-palmitoyl glycerol
16:0	palmitic acid
18:1n-9	oleic acid
18:2n-6	linoleic acid
18:3n-3	α-linolenic acid
20:4n-6	arachidonic acid
CB1	cannabinoid receptor 1
CCK	cholecystokinin
FA	fatty acid(s)
FFA	free fatty acid(s)
GLP-1	glucagon-like peptide-1
GPR120	G-protein coupled receptor 120
LDH	lactate dehydrogenase
MAGL	monoacylglycerol lipase
NEFA	nonesterified fatty acid(s)
TG	triacylglycerol(s)

Introduction

The gastrointestinal endocrine cells scattered along the digestive tract accurately capture the chemical information of nutrients and non-nutrients; they release gut hormones into the body. Gut hormones mediate nutrient and non-nutrient signals to regulate gastrointestinal functions, including secretion, motility, digestion, and absorption. Cholecystokinin (CCK) is a gut hormone produced by enteroendocrine 'I cells' located in the upper small intestine (Dockray 2012; Rehfeld 2000). CCK regulates gallbladder contraction, pancreatic enzyme secretion, gastric emptying, and food intake (Liddle 1997).

Dietary lipid-derived triacylglycerols (TG) are hydrolyzed to fatty acids (FA) and 2-monoacylglycerols (2-MAG) in the small intestine. FA with chain lengths of more than 12 carbons strongly stimulate CCK secretion (Feltrin *et al.* 2004; Hira *et al.* 2004; McLaughlin *et al.* 1999; McLaughlin *et al.* 1998; Sidhu *et al.* 2000). However, the effect of 2-MAG on CCK secretion remains unclear.

2-oleoyl glycerol (2-OG), a 2-MAG, and oleoylethanolamide, an ethanolamide derived from oleic acid, stimulate the secretion of another gut hormone, glucagon-like peptide-1 (GLP-1) via GPR119 (Lauffer *et al.* 2009; Hansen *et al.* 2011; Hansen *et al.* 2012). GPR119 is one of the GPCRs expressed in enteroendocrine cells, such as I and L cells, in the human and rodent intestinal tissue (Chu *et al.* 2008; Lauffer *et al.* 2009; Sykaras *et al.* 2012), and the murine enteroendocrine cell lines, STC-1 and GLUTag (Chu *et al.* 2008). However, it is unclear whether GPR119 is involved in other sensing mechanisms of 2-MAG and/or CCK secretion.

Some studies have examined the effects of 2-MAG on food intake. Jejunal administration of 2-OG reduced food intake in rats without causing diarrhea (Okuma *et al.* 2016). Inhibition or deficiency of monoacylglycerol acyltransferase 2, which catalyzes

65 the conversion of 2-MAG to diacylglycerol, reduced food intake in mice fed a high-fat
66 diet (Nelson *et al.* 2014; Okuma *et al.* 2015). Furthermore, overexpression of
67 monoacylglycerol lipase (MAGL) in mice, which specifically hydrolyzes 2-MAG into
68 FFA and glycerol in the small intestine, induced hyperphagia compared to normal mice
69 (Chon *et al.* 2012). These results suggest that 2-MAG is involved in appetite suppression,
70 possibly through CCK and/or GLP-1.
71 In the present study, we examined whether 2-MAG could induce CCK secretion
72 in CCK-producing STC-1 cells. CCK secretion was examined in response to four
73 different commercially available 2-MAG, and the cellular mechanism was investigated.
74

75 **Materials and Methods**

76 *Materials*

77 Cell culture consumables (Dulbecco's modified Eagle's medium [DMEM] and
78 fetal bovine serum [FBS]) were purchased from Invitrogen (Carlsbad, CA, USA). HEPES
79 and FA were purchased from Sigma (St. Louis, MO, USA). 2-MAG, 2-arachidonoyl
80 glycerol ether (2-AG ether), 1-arachidonoyl glycerol (1-AG), and a G-protein coupled
81 receptor 120 (GPR120) antagonist, AH7614, were purchased from Cayman Chemical
82 (Ann Arbor, MI, USA). Glycerol was purchased from Tokyo Chemical Industry (Tokyo,
83 Japan). A selective CB1 antagonist, SR141716A, was purchased from Tocris Bioscience
84 (Ellisville, MO, USA). Unless otherwise specified, all other reagents were purchased
85 from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).
86

87 *Cell culture*

88 STC-1 cells (a gift from D. Hanahan, University of California, San Francisco,

CA, USA) were grown in DMEM (Invitrogen, Cat. No. 12100-038) supplemented with 10% FBS, 50 IU/mL penicillin, and 500 µg/mL streptomycin in a humidified 5% CO_2 atmosphere at 37°C. Cells were routinely subcultured by trypsinization upon reaching 80-90% confluence. Cells at passage numbers 30-50 were used for the experiments.

CCK secretion study in STC-1 cells

STC-1 cells were seeded in 48-well culture plates at a density of 1.5×10^5 cells/well and grown for 2-3 days until reaching 80-90% confluency. Cells were washed twice with HEPES buffer to remove the culture medium and then exposed to the test agents dissolved in HEPES buffer containing 0.1 or 0.2% ethanol (to prepare monoacylglycerol and FA solutions) for 60 min at 37°C. The HEPES buffer (pH 7.4) had the following composition: 140 mM NaCl, 4.5 mM KCl, 20 mM HEPES, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM D-glucose. Following incubation, the supernatants were collected into 1.5 mL tubes and centrifuged at 800 × g for 5 min at 4°C to remove the remaining cells. The supernatants were then stored at –30°C until CCK concentration was measured using a commercially available enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Belmont, CA, USA). The primary antiserum provided in this kit cross-reacts (100%) with sulfated and non-sulfated CCK (26-33), CCK-33 (porcine), caerulein, gastrin-1 (human), and big gastrin-1 (human). The antiserum also cross-reacts (12.6%) with CCK (30-33); however, it shows 0% cross-reaction with pancreatic polypeptide (human) and vasoactive intestinal peptide (including human, porcine, and rat). Because STC-1 cells do not express detectable gastrin levels (McLaughlin *et al.* 1998), an EIA kit was selected in which the antibody cross-reacts with gastrin. The coefficients of the intra- and inter-assay variations were < 5% and < 14%, respectively.

Measurement of cytotoxicity in STC-1 cells

The cytotoxic effects on STC-1 cells were determined by measuring the release of lactate dehydrogenase (LDH) from the cells into the supernatant after the cells were exposed to the test agents as described above. LDH was measured using a cytotoxicity detection kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Cytotoxicity was calculated as the relative release (%) of LDH after exposure to the test agents compared to the total LDH (100%) released upon treatment with the lysis reagent supplied in the kit.

Measurement of nonesterified FA concentration in the supernatant

STC-1 cells were exposed to the test agents as described above, and the supernatants were collected every 15 min for 60 min. Nonesterified FA (NEFA) concentrations in the supernatants were measured using a NEFA-C kit (Wako Pure Chemical Corporation, Osaka, Japan) according to the manufacturer's instructions.

RT-PCR

RNA was isolated from STC-1 cells cultured in 75 cm^2 tissue culture flasks and from BALB/c mouse (CLEA Japan, Tokyo, Japan) tissues (duodenum, jejunum, ileum, and colon) using an RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The study was approved by the Hokkaido University Animal Committee, and the animals were maintained following the guidelines for the care and use of laboratory animals of Hokkaido University. cDNA was prepared from 1 μg RNA using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka,

Japan) and subjected to PCR using primers based on the mouse CB1 mRNA sequence (GenBank accession number NM001355020; forward primer 5'-CCACCTTCCGTACCATCACC-3', reverse primer 5'-AACCAACGGGGAGTTGTCTC-3') and the mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA sequence (GenBank accession number NM008084; forward primer 5'-TCACCACCATGGAGAAGGC-3', reverse primer 5'-GCTAAGCAGTTGGTGGTGCA-3'). PCR conditions were as follows: 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 63.3°C for 30 s, and 72°C for 30 s. PCR products were separated by 1.5% agarose gel electrophoresis and visualized by Midori Green Advance DNA stain (NIPPON Genetics, Tokyo, Japan).

Statistical analyses

The results are expressed as the mean ± SEM. Statistical analyses were performed using JMP Pro version 14.0.0 software (SAS Institute, Inc., Cary, USA). Statistically significant difference was determined using one-way ANOVA followed by Tukey-Kramer's test. Significant effects of lipids (2-MAG and/or FA), antagonists, and their interactions were assessed using two-way ANOVA. In all analyses, $p < 0.05$ was considered statistically significant.

Results

2-AG and arachidonic acid potently stimulate CCK secretion from STC-1 cells

We first explored the release of CCK in response to four types of commercially available 2-MAG (i.e., 2-palmitoyl glycerol: 2-PG, 2-oleoyl glycerol: 2-OG, 2-linoleoyl glycerol: 2-LG, and 2-arachidonoyl glycerol: 2-AG). Of these, 2-AG induced a significant

161 increase in CCK secretion at doses of 100 and 200 μM (Fig. 1a and b). The CCK secretory
162 activity of 2-AG was as strong as that of α-linolenic acid (18:3n-3), which is known to
163 promote CCK secretion in STC-1 cells (Tanaka *et al.* 2008).

164 Next, we examined the CCK secretory activity of the FA comprising these 2-
165 MAG (i.e., palmitic acid: 16:0, oleic acid: 18:1n-9, linoleic acid: 18:2n-6, and arachidonic
166 acid: 20:4n-6). Among the FA, 20:4n-6 significantly increased CCK secretion at 100 μM,
167 with higher potency than 18:3n-3 (Fig. 1c).

168 Both 2-AG and 20:4n-6 stimulated CCK secretion in a dose-dependent manner
169 (Fig. 1d and e). Significant increases in CCK secretion compared to vehicle treatment
170 were observed with 2-AG at more than 50 μM, while with 20:4n-6 at more than 100 μM.

171 LDH release was measured to examine whether the exposed compound-induced
172 cell lysis caused CCK release. None of the tested compounds induced LDH release,
173 regardless of the concentration or structure (Fig. S1).

174

175 *Structural specificity of 2-AG-induced CCK secretion*

176 Intracellular calcium mobilization induced by FA triggers CCK secretion in
177 enteroendocrine cells (Hira *et al.* 2004; McLaughlin *et al.* 1998). In previous studies, 2-
178 AG elicited a rapid increase in intracellular Ca^{2+} concentration, while several structural
179 analogs of 2-AG, such as 1-AG, were less active than 2-AG (Sugiura *et al.* 1997; Sugiura
180 *et al.* 1996). To investigate the structure-activity relationship, we compared the effects of
181 various structurally related compounds (i.e., 2-AG ether and 1-AG) and hydrolysates (i.e.,
182 glycerol and 20:4n-6) with 2-AG. As shown in Fig. 2, 2-AG and 20:4n-6 significantly
183 increased CCK secretion in STC-1 cells; however, 2-AG ether, 1-AG, and glycerol did
184 not. None of the tested compounds caused LDH release (Fig. S2).

185

186 *Verification of the liberation of NEFA from 2-AG in STC-1 cells*

187 To examine whether 20:4n-6 was liberated from 2-AG during secretion study in
188 STC-1 cells, we measured NEFA concentrations in the supernatant. As shown in Fig. 3,
189 NEFA concentration did not increase in the STC-1 cell supernatant during exposure to 2-
190 AG and the vehicle. 20:4n-6 dosed at 100 μM resulted in a decrease in NEFA
191 concentration by 60 μM over time. In the absence of STC-1 cells, NEFA concentration in
192 the solution containing 100 μM 20:4n-6 added into the well did not decrease (data not
193 shown), suggesting that free 20:4n-6 was taken up by the cells during the incubation.

194

195 *Involvement of CB1*

196 2-AG is known as an endogenous cannabinoid and an endogenous ligand for
197 both cannabinoid receptors CB1 and CB2 (Ueda *et al.* 2011; Gendaszewska-Darmach *et*
198 *al.* 2019). CB1 is expressed in mouse duodenal CCK-producing cells (Argueta *et al.* 2019;
199 Sykaras *et al.* 2012). We detected single bands of expected DNA size (182 bp) for CB1
200 in STC-1 cells and mouse intestinal tissues by conventional PCR (Fig. 4a).

201 We next performed a CCK secretion study in the presence of SR141716A, a CB1
202 selective antagonist. Under treatment with SR141716A at 10 and 30 nM, 2-AG-induced
203 CCK secretion was diminished (Fig. 4b), whereas 20:4n-6-induced CCK secretion was
204 not affected by the treatment (Fig. 4c).

205

206 *Involvement of the FA receptor GPR120*

207 We examined the involvement of the FA receptor GPR120 (Tanaka *et al.* 2008;
208 Ulven and Christiansen 2015) as a potential sensor for 2-AG because 20:4n-6 reportedly

209 acts on GPR120 (Mobraten *et al.* 2013; Villegas-Comonfort *et al.* 2017). Treatment with
210 a GPR120 antagonist, AH7614, completely diminished CCK secretion induced by 18:3n-
211 3, which is known to stimulate CCK secretion via GPR120 (Tanaka *et al.* 2008), while 2-
212 AG-induced CCK secretion was not reduced (Fig. 5a). 20:4n-6-induced CCK secretion
213 was partially attenuated by this treatment (Fig. 5b).
214
215 **Discussion**
216 Although FA induce CCK secretion in a carbon chain length-dependent manner
217 (Feltrin *et al.* 2004; Hira *et al.* 2004; McLaughlin *et al.* 1999; McLaughlin *et al.* 1998;
218 Sidhu *et al.* 2000), the effect of 2-MAG on CCK secretion has not been clarified. In the
219 present study, we examined whether 2-MAG could stimulate CCK secretion in the murine
220 CCK-producing enteroendocrine cell line, STC-1. We demonstrated that a specific 2-
221 MAG, 2-AG, potently stimulated CCK secretion through the cannabinoid receptor CB1.
222 In the present study, we tested four commercially available 2-MAG (i.e., 2-PG,
223 2-OG, 2-LG, and 2-AG) on their CCK-releasing activities. Of them, 2-PG, 2-OG, and 2-
224 LG, especially 2-OG, are known agonists of GPR119 (Hansen *et al.* 2012). 2-OG
225 stimulates the secretion of incretin hormones, GLP-1 and GIP, via GPR119 in humans,
226 animals, and the murine enteroendocrine cell line, GLUTag (Hansen *et al.* 2011; Hansen
227 *et al.* 2012; Okuma *et al.* 2016; Hassing *et al.* 2016). Therefore, it was possible that all
228 these 2-MAG could stimulate CCK secretion via GPR119. However, 2-OG, 2-PG, and 2-
229 LG did not stimulate CCK secretion in STC-1 cells (Fig. 1a and b). The concentrations
230 of these 2-MAG in the present study were probably insufficient to activate GPR119
231 expressed in STC-1 cells because STC-1 cells reportedly have lower expression of
232 GPR119 than GLUTag cells (Chu *et al.* 2008). Among the 2-MAG tested, only 2-AG at

a dose greater than 50 μM stimulated the CCK secretion (Fig. 1). Although 20:4n-6 also stimulated CCK secretion, it did so at a dose greater than 100 μM, indicating that the CCK-releasing activity of 2-AG is more potent than that of 20:4n-6. Furthermore, these results suggest that 2-AG and 20:4n-6 stimulate CCK secretion via distinct mechanisms.

Comparison of 2-AG related compounds (2-AG ether, 1-AG, and glycerol) shown in Fig. 2 revealed that the ester bond of 2-AG and its binding to the *sn*-2 but not the *sn*-1 position in acylglycerol is essential for 2-AG-induced CCK secretion.

2-AG is degraded by hydrolytic enzymes such as MAGL (Dinh *et al.* 2002a; Dinh *et al.* 2002b). MAGL is expressed in the brain and intestinal epithelium and functions both in the cytosol and the cell membrane (Blankman *et al.* 2007). NEFA concentration did not increase in STC-1 cell supernatant during exposure to 2-AG (Fig. 3), suggesting that 2-AG is hardly hydrolyzed on the extracellular surface of STC-1 cells. In contrast, we might have failed to detect 20:4n-6 in the supernatant because 20:4n-6 liberated from 2-AG was immediately taken up into the cell. As shown in Fig. 3, free 20:4n-6 added to the supernatant gradually decreased by 60 μM during the 60 min incubation. These results demonstrate that STC-1 cells can uptake or degrade approximately 40 μM of extracellular 20:4n-6 within 60 min. As shown in Fig. 1e, 50 μM 20:4n-6 was not sufficient to induce CCK secretion. If more than 50 μM of 20:4n-6 was liberated from 2-AG during the secretion study, we should be able to detect an increase in NEFA in the supernatant. Additionally, a previous study reported no apparent change in intracellular Ca^{2+} concentration with 20:4n-6 below 30 μM (Sugiura *et al.* 1996). These observations support the notion that 2-AG-induced CCK secretion is independent of 20:4n-6 liberated from 2-AG.

RT-PCR confirmed the expression of CB1 mRNA in STC-1 cells (Fig. 4a) as

257 well as in mouse tissues. CB1 is a specific receptor for endocannabinoids, such as 2-AG
258 and anandamide (Ueda *et al.* 2011; Pacher *et al.* 2006). Our results (Fig. 4b and 5a)
259 suggest that CB1 functions as a 2-AG sensor to release CCK in CCK-producing
260 enteroendocrine cells because 2-AG-induced CCK secretion was effectively attenuated
261 by the CB1 antagonist, but not by the GPR120 antagonist. In contrast, 20:4n-6-induced
262 CCK secretion was diminished by the GPR120 antagonist (Fig. 5b), but not by the CB1
263 antagonist (Fig. 4c). These results demonstrate that 2-AG itself stimulates CCK secretion
264 by acting on CB1, but not through 20:4n-6 released from 2-AG. 2-AG ether and 1-AG
265 are also known as agonists for CB1 but did not induce CCK secretion, probably because
266 their agonistic potency for CB1 is 10- to 100-fold weaker than 2-AG (Sugiura *et al.* 1997;
267 Sugiura *et al.* 1996; Sugiura *et al.* 1999; Hanuš *et al.* 2001).

268 Activation of CB1 is reportedly involved in the regulation of appetite
269 enhancement, energy homeostasis, and gastrointestinal function (Izzo *et al.* 1999;
270 Krowicki *et al.* 1999; Landi *et al.* 2002; Marzo *et al.* 2008; Pertwee 2001; Argueta and
271 DiPatrizio 2017; DiPatrizio *et al.* 2011). A previous study demonstrated the inhibitory
272 effect of CB1 agonist on corn oil-induced CCK secretion and CCK-mediated satiety
273 effects in mice (Argueta *et al.* 2019). These results are mostly obtained by experiments
274 using pharmacological agonists, such as WIN 55,212-2 and CP 55,940. However, it
275 remains unclear whether 2-AG derived from dietary lipids stimulates CCK secretion by
276 acting on the apical CB1 of I cells. Since both 2-AG and 20:4n-6 potently stimulated CCK
277 secretion in the present study, TG rich in 20:4n-6 could effectively exert physiological
278 effects, such as satiety induction and suppression of gastric emptying, by enhancing CCK
279 secretion *in vivo*.

280 The main dietary sources of 20:4n-6 are animal foods, such as meat, poultry,

eggs, fish, and dairy foods (Li *et al.* 1998; Taber *et al.* 1998; Komprda *et al.* 2005; Abedi and Sahari 2014; Kawashima 2019; Tallima and El Ridi 2018). Membrane phospholipids containing dietary-derived 20:4n-6 could be used for endogenous 2-AG production by the action of phospholipase C and subsequent diacylglycerol lipase (Ueda *et al.* 2011). In addition, ingestion of meat products and egg yolks may result in the formation of 2-AG due to the action of phospholipase A_1 in the gastrointestinal tract and subsequent phosphatase or phospholipase C in the cells (Aoki *et al.* 2007; Ueda *et al.* 1993; Nakane *et al.* 2002). Further studies are required to verify these possibilities and demonstrate whether dietary lipid-derived or endogenous 2-AG induces physiological effects through the induction of CCK secretion and the involvement of CB1 in nutrient sensing in enteroendocrine cells *in vivo*.

In conclusion, by examining various 2-MAG, we identified 2-AG as a potent stimulator of CCK secretion in the murine enteroendocrine cell line, STC-1. The structure-activity relationship was clarified by comparison with 20:4n-6, 1-AG, and 2-AG ether. Furthermore, we demonstrated that CB1 mediates 2-AG-induced CCK secretion. These results reveal a novel 2-MAG-sensing mechanism in enteroendocrine cells and suggest that dietary lipid-derived 2-AG or endogenous 2-AG causes physiological effects via CCK secretion.

Authorship

K.O. and T.H. conceived and designed the study and wrote the first draft of the manuscript, K.O. carried out the experiments, K.O. and T.H. analyzed the data. All authors contributed to and approved the final draft of the manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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Figure legends

Fig. 1 Secretion of CCK in response to various 2-MAG and FA. STC-1 cells cultured in 48-well plates were exposed to (a) 100 μM, (b) 200 μM 2-MAG, and 200 μM 18:3n-3, (c) various FA, various concentrations of (d) 2-AG, or (e) 20:4n-6 for 60 min at 37°C. Values are expressed as the mean ± SEM (n=3). Values not sharing a common letter are significantly different ($p < 0.05$, Tukey's test). 2-PG, 2-palmitoyl glycerol; 2-OG, 2-oleoyl glycerol; 2-LG, 2-linoleoyl glycerol; 2-AG, 2-arachidonoyl glycerol; 18:3n-3, α-linolenic acid; 16:0, palmitic acid; 18:1n-9, oleic acid; 18:2n-6, linoleic acid; 20:4n-6, arachidonic acid

Fig. 2 Effect of compounds structurally related to 2-AG on CCK secretory activity. STC-1 cells cultured in 48-well plates were exposed to 100 μM 2-AG, structurally related compounds (i.e., 2-AG ether and 1-AG), glycerol, and 20:4n-6. Values are expressed as the mean ± SEM (n=3). Values not sharing a common letter are significantly different ($p < 0.05$, Tukey's test). 2-AG, 2-arachidonoyl glycerol; 2-AG ether, 2-arachidonoyl glycerol ether; 1-AG, 1-arachidonoyl glycerol; 20:4n-6: arachidonic acid

Fig.3 Changes in NEFA concentrations in supernatant during the secretion study in STC-1 cells. STC-1 cells cultured in 48-well plates were exposed to 2-AG (100 μM) or 20:4n-6 (100 μM) at 37°C. The supernatants were collected every 15 min until 60 min. The concentration of NEFA in the supernatants was measured. Values are expressed as the mean ± SEM (n=3). 2-AG, 2-arachidonoyl glycerol; 20:4n-6, arachidonic acid

Fig. 4 Expression of CB1 in STC-1 cells and mouse intestine, and effects of CB1

antagonist on CCK secretion induced by 2-AG and 20:4n-6. (a) Total RNA was extracted from STC-1 cells and mouse intestine (duodenum, jejunum, ileum, and colon) and then subjected to RT-PCR with specific CB1 or GAPDH primers. PCR products were separated in agarose gel and visualized by Midori Green Advance DNA stain. STC-1 cells cultured in 48-well plates were exposed to (b) 2-AG (100 μM) or (c) 20:4n-6 (100 μM) in the presence of a CB1 antagonist (SR141716A) for 60 min at 37°C. Values are expressed as the mean ± SEM (n=3). Values not sharing a common letter are significantly different ($p < 0.05$, Tukey's test). P values of two-way ANOVA for lipid (2-AG or 20:4n-6), antagonist (SR141716A), and their interactions are shown in the panels. 2-AG, 2-arachidonoyl glycerol; 20:4n-6, arachidonic acid

Fig. 5 Effects of GPR120 antagonist on CCK secretion induced by 2-AG and 20:4n-6. STC-1 cells cultured in 48-well plates were exposed to (a) 2-AG (100 μM) or (b) 20:4n-6 (100 μM) in the presence of a GPR120 antagonist (AH7614) for 60 min at 37°C. Values are expressed as the mean ± SEM (n=3). Values not sharing a common letter are significantly different ($p < 0.05$, Tukey's test). P values of two-way ANOVA for lipids (18:3n-3 and 2-AG, or 20:4n-6), antagonist (AH7614), and their interactions are shown in the panels. 18:3n-3, α-linolenic acid; 2-AG, 2-arachidonoyl glycerol; 20:4n-6, arachidonic acid

Fig. 1

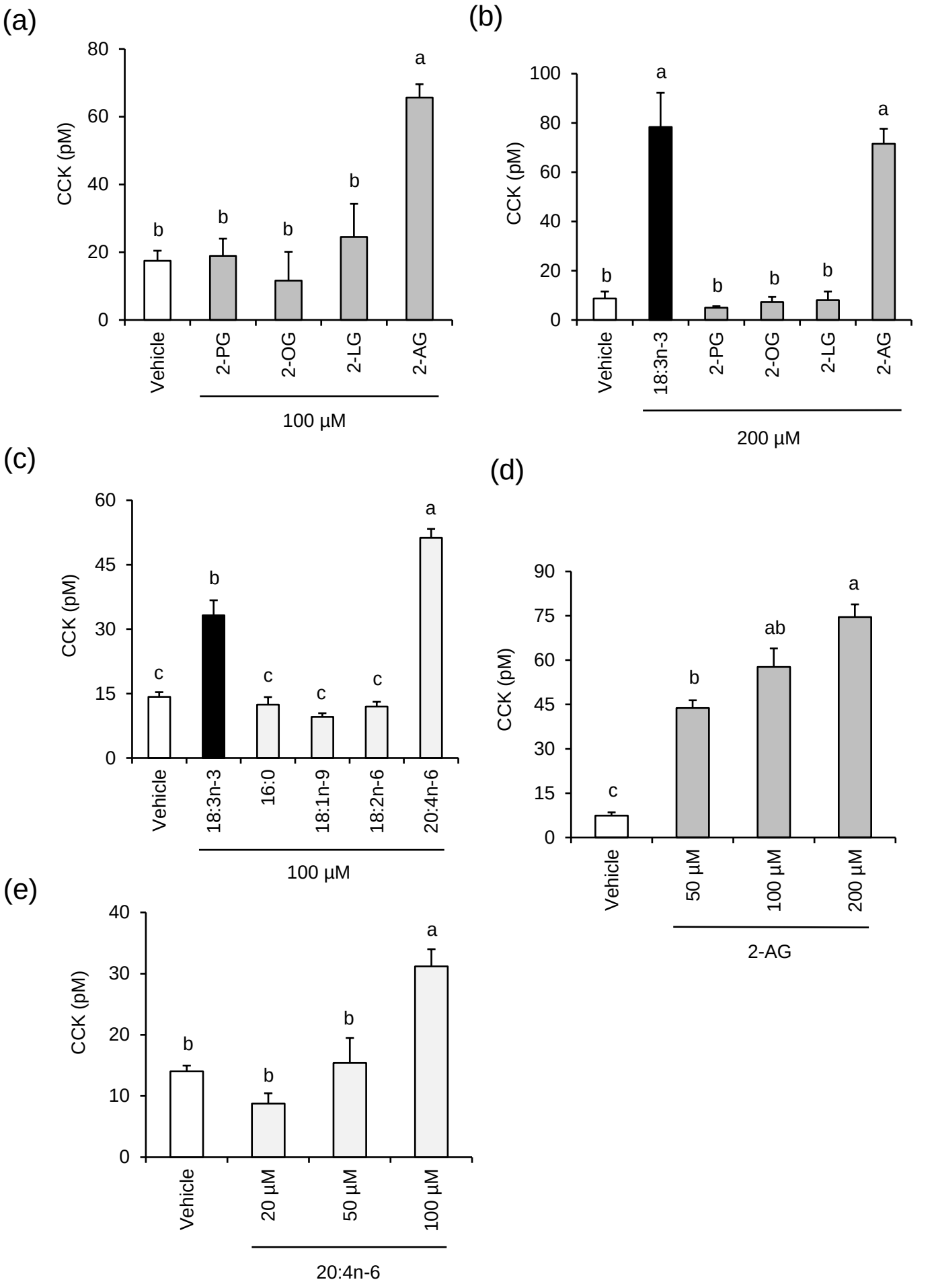

(a)
CCK (pM)
80
60
40
20
0
b
b
b
b
a
Vehicle
2-PG
2-OG
2-LG
2-AG
100 μM
(b)
CCK (pM)
100
80
60
40
20
0
b
a
b
b
b
a
Vehicle
18:3n-3
2-PG
2-OG
2-LG
2-AG
200 μM
(c)
CCK (pM)
60
45
30
15
0
c
b
c
c
c
a
Vehicle
18:3n-3
16:0
18:1n-9
18:2n-6
20:4n-6
100 μM
(d)
CCK (pM)
90
75
60
45
30
15
0
c
b
ab
a
Vehicle
50 μM
100 μM
200 μM
2-AG
(e)
CCK (pM)
40
30
20
10
0
b
b
b
a
Vehicle
20 μM
50 μM
100 μM
20:4n-6

Fig. 2

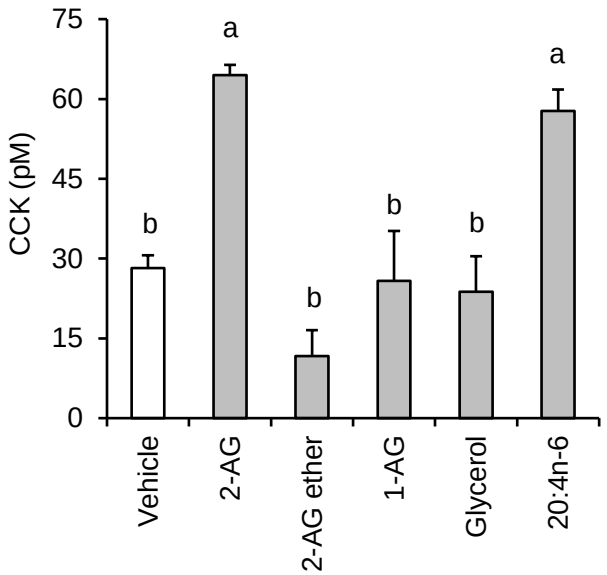

CCK (pM)
75
60
45
30
15
0
b
a
b
b
b
a
Vehicle
2-AG
2-AG ether
1-AG
Glycerol
20:4n-6

Fig. 3

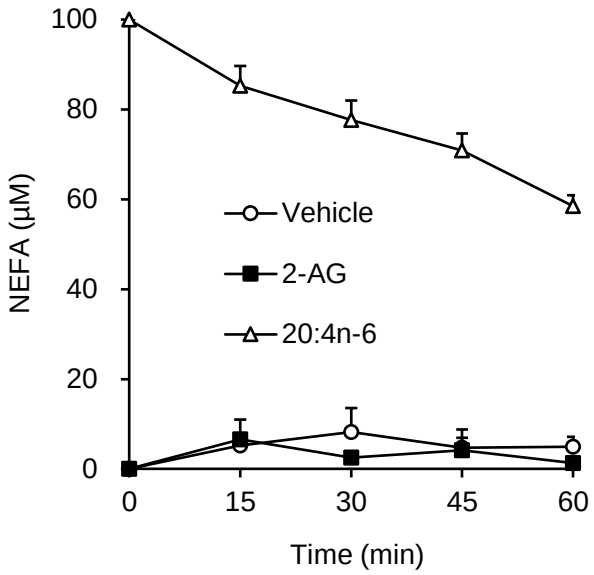
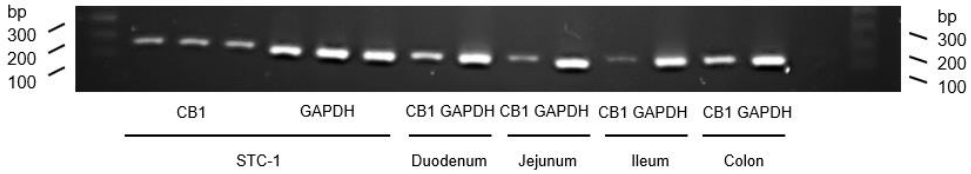
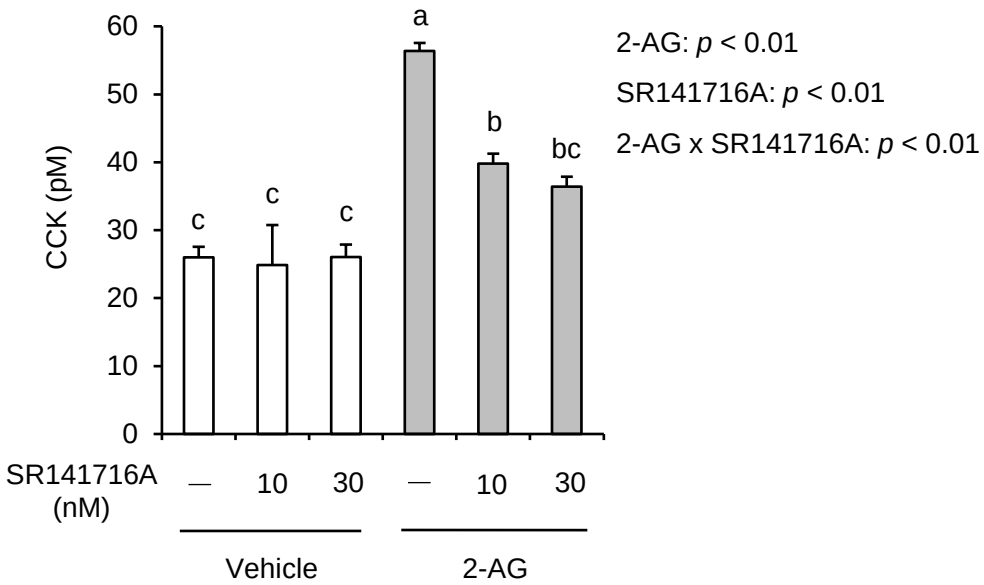


Fig. 4

(a)


bp
300
200
100
bp
300
200
100
CB1
GAPDH
CB1 GAPDH
CB1 GAPDH
CB1 GAPDH
CB1 GAPDH
STC-1
Duodenum
Jejunum
Ileum
Colon

(b)


CCK (pM)
60
50
40
30
20
10
0
a
b
bc
c
c
c
SR141716A (nM)
—
10
30
—
10
30
Vehicle
2-AG
2-AG: p < 0.01
SR141716A: p < 0.01
2-AG x SR141716A: p < 0.01

(c)

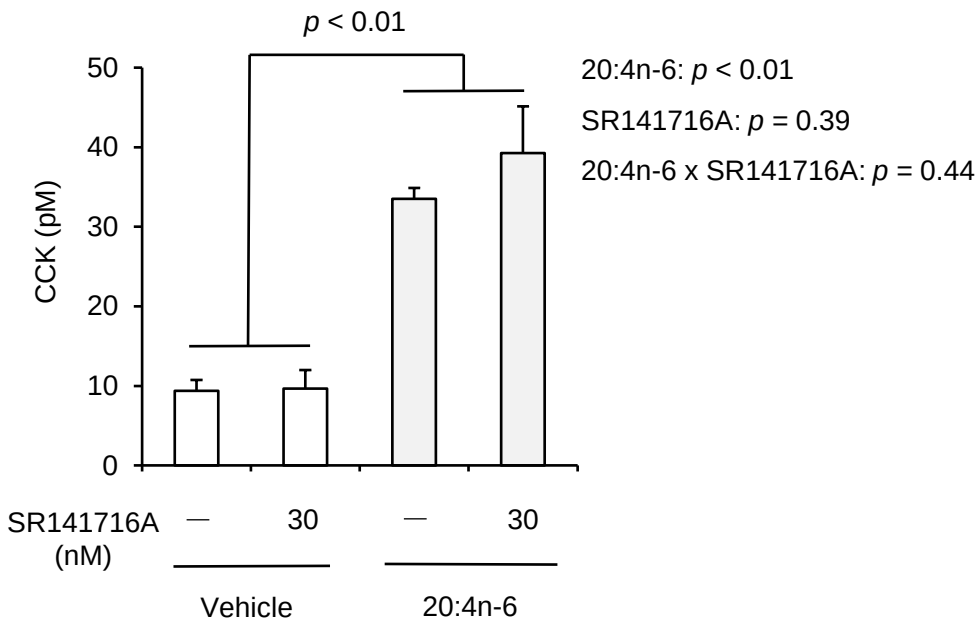
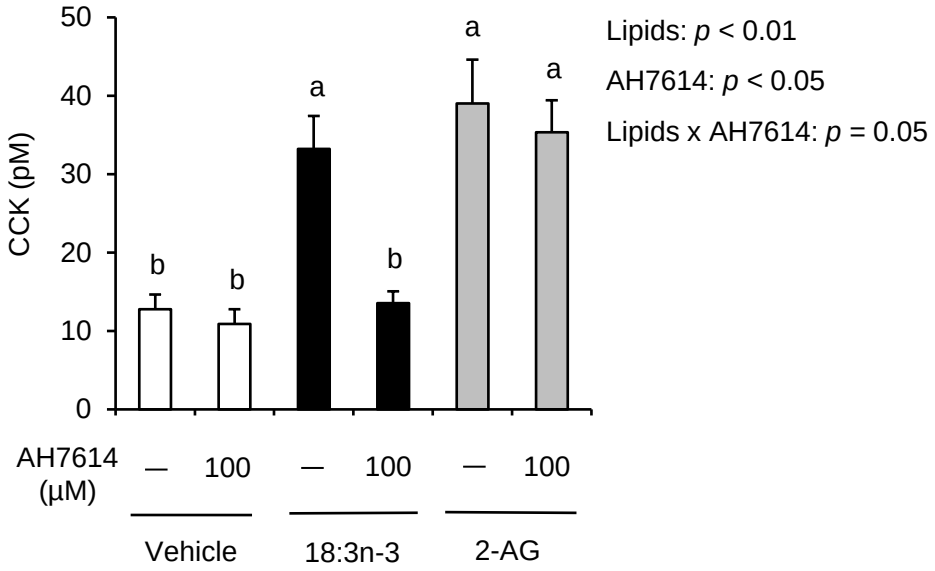
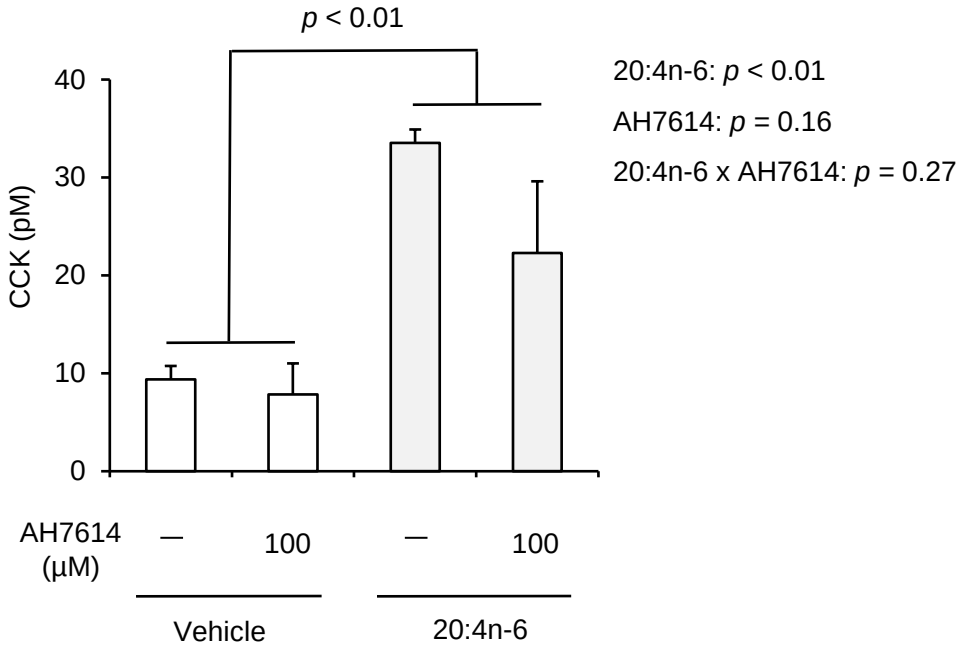

p < 0.01
CCK (pM)
50
40
30
20
10
0
SR141716A (nM)
—
30
—
30
Vehicle
20:4n-6
20:4n-6: p < 0.01
SR141716A: p = 0.39
20:4n-6 x SR141716A: p = 0.44

Fig. 5

(a)


50
40
30
20
10
0
CCK (pM)
b
b
a
b
a
a
AH7614 (μM)
—
100
—
100
—
100
Vehicle
18:3n-3
2-AG
Lipids: p < 0.01
AH7614: p < 0.05
Lipids x AH7614: p = 0.05

(b)


p < 0.01
40
30
20
10
0
CCK (pM)
AH7614 (μM)
—
100
—
100
Vehicle
20:4n-6
20:4n-6: p < 0.01
AH7614: p = 0.16
20:4n-6 x AH7614: p = 0.27

Title

2-arachidonoyl glycerol potently induces cholecystokinin secretion in murine enteroendocrine STC-1 cells via cannabinoid receptor CB1

Journal

Lipids

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